



# Current management of melanoma patients with nodal metastases

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## Abstract

The management of melanoma patients with nodal metastases has undergone dramatic changes over the last decade. In the past, the standard of care for patients with a positive sentinel lymph node biopsy (SLNB) was a completion lymph node dissection (CLND), while patients with palpable macroscopic nodal disease underwent a therapeutic lymphadenectomy in cases with no evidence of systemic spread. However, studies have shown that SLN metastases present as a spectrum of disease, with certain SLN-based factors being prognostic of and correlated with outcomes. Furthermore, the results of key clinical trials demonstrate that CLND provides no survival benefit over nodal observation in positive SLN patients, while other clinical trials have shown that adjuvant immune checkpoint inhibitor therapy or targeted therapy after CLND is associated with a recurrence-free survival benefit. Given the efficacy of these systemic therapies in the adjuvant setting, these agents are now being evaluated and utilized as neoadjuvant treatments in patients with regionally-localized or resectable metastatic melanoma. Multiple options now exist to treat melanoma patients with nodal disease, and determining the best treatment course for a particular case requires an in-depth knowledge of current data and an informed discussion with the patient. This review will provide an overview of the various options for treating melanoma patients with nodal metastases and will discuss the data that supported the development of these treatment options.

**Keywords** Melanoma · Sentinel lymph node metastasis · Completion lymph node dissection · Neoadjuvant systemic therapy · Adjuvant systemic therapy · Immune checkpoint inhibitor and targeted therapy

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## Overall introduction

Dale Han, MD

The presence of nodal metastases in patients with melanoma portends more aggressive tumor biology and is associated with worse survival. Despite the poorer prognosis seen in patients with nodal disease, the standard of care in the past for patients with microscopic nodal metastases found through sentinel lymph node biopsy (SLNB) was a completion lymph node dissection (CLND), while for patients with palpable macroscopic nodal metastases, the standard of care was a therapeutic lymph node dissection (TLND) in cases with no distant disease. The primary reason for the surgical management of nodal metastases was to provide regional disease control given that there were no effective systemic therapy options available in the past, although the survival benefit provided by these procedures was questioned. However, due to the results of recent key clinical trials and the development of efficacious systemic therapies,

the management of melanoma patients with nodal disease has undergone dramatic changes over the last decade.

The results of recent clinical trials have demonstrated that CLND overall provides no survival benefit over nodal observation in positive SLN patients. However, other studies have shown that SLN metastases present as a spectrum of disease, with certain SLN-based factors being prognostic of and correlated with outcomes. Furthermore, it is unknown if a certain small subset of positive SLN patients may gain a benefit from CLND. Other clinical trials have shown that adjuvant immune checkpoint inhibitor therapy or targeted therapy given after CLND is associated with a recurrence-free survival benefit. Given the efficacy of these systemic therapies in the adjuvant setting, these agents are now being evaluated and utilized as neoadjuvant treatments in patients with regionally-localized or resectable metastatic melanoma, particularly in cases that have macroscopic nodal metastases.

Multiple therapeutic options now exist to treat melanoma patients with nodal disease, and determining the best treatment course for a particular case requires a comprehensive knowledge of current data and an informed discussion with the patient, taking into account a number of factors. This review will provide an overview of the various options for treating melanoma patients with nodal metastases and will discuss the data that supported the development of these treatment options. Dr. van Akkooi will first examine the spectrum of presentation for SLN micrometastasis and will show how specific characteristics of the SLN micrometastasis correlate with outcomes in melanoma patients. Dr. Reintgen will then discuss surgical management options in melanoma patients with a positive SLN and whether a CLND should be performed for SLN metastases or if specific positive SLN patients should be offered or selected for CLND. Moreover, efficacious systemic therapies for metastatic melanoma are now available, and Drs. Wang and Kim will review systemic adjuvant therapy for melanoma with regional nodal metastases, while Drs. Straker III, Shannon, and Karakousis will discuss the development of neoadjuvant systemic therapy for clinical stage III melanoma.

## Spectrum of micrometastasis in melanoma sentinel lymph nodes

Alexander C.J. van Akkooi, MD, PhD

Historically, the 5-year survival prognosis of patients with a positive SLN for melanoma has been reported to be around 70%. This was the case for the American Joint Committee on Cancer (AJCC) 7th edition pooled database by Balch et al. in 2009 [1]. This is similar to reports from large institutional databases and from prospective trials, like the Multicenter Selective Lymphadenectomy Trial (MSLT)'s [2–5]. However, not all positive SLN metastases can be

considered the same prognostically. A SLNB is not a simple positive or negative result, and there is a gradient from very tiny to very large SLN tumor burden, with correspondingly increasing risks.

The first reason to study SLN tumor burden was to see if this could be used to appropriately select patients to undergo or to forgo a CLND. Later, SLN tumor burden was studied to see if it could be used to select high-risk patients for adjuvant systemic therapy trials. Many different SLN tumor burden factors have been proposed and examined, with and without considering primary tumor factors [6]. Ranieri et al. and Carlson et al. were two of the first to look at a threshold of 3 and 2 mm, respectively [7, 8]. Other factors that have been proposed included infiltration from the capsule, surface area of node involved [either as a percentage of node involved (%) or as square area (mm<sup>2</sup>)], the microanatomic location, and the absence of presence of extra-capsular extension (ECE) [9–23]. However, with all of these criteria, the difficulty is that we use 2 dimensional pathology measurements, whereas both a SLN and the metastases are 3 dimensional. Moreover, lymph nodes and metastases are never straight or square but are instead curved and irregular. Thus, a simple 2 dimensional measurement will never do justice to the actual situation.

One of the initial and most frequently used SLN tumor burden classification systems was the Starz classification, which evaluates the infiltration of the SLN metastasis from the capsule inwards (and originally also the amount of involved levels) [21, 22]. Increasing infiltration corresponded with increasing risk of positive nodes in the CLND specimen and worse survival. The difficulty in implementing this in practice is that lymph nodes are not square but curved, and it can be a challenge to see where the capsule is closest. Moreover, sometimes the capsule cannot be reliably determined and the question arises as to how to measure in cases of trabeculae? Finally, if one includes measurements on multiple levels, thus more measurements, the chance of more inter-observer variability increases.

Another classification often used was the micro-anatomic location according to Dewar et al. [11]. They showed that patients with exclusively subcapsular metastases had no chance for additional positive nodes in the CLND specimen and had an excellent survival. However, it is somewhat difficult to determine when a lesion is still subcapsular or when it is no longer confined to the subcapsular space and starts infiltrating into the parenchyma. Also, when is the category of “multifocal” appropriate? Does this start when there are 2 lesions in a SLN (which is often true for melanoma in which there is not a single site of metastasis in a SLN)?

Finally, the largest diameter of the largest lesion is the single most frequently used SLN tumor burden assessment. The Rotterdam criteria, developed by van Akkooi et al. demonstrated that increasing SLN tumor burden

corresponded to worse survival and to a higher chance for non-SLN metastases in the CLND [24, 25]. Patients with tiny metastases ( $\leq 0.1$  mm) had an excellent prognosis, similar to SLN-negative patients, and never had additional nodal involvement [24, 25]. Patients with larger metastases ( $\geq 1$  mm) had a poor prognosis, similar to patients with macroscopic (palpable) nodal disease. The Rotterdam criteria were validated in a large series of cases from 8 European Organization for Research and Treatment of Cancer (EORTC) sites and thereafter was adopted by EORTC [26]. This was validated once more with patients from the Melanoma Institute Australia (MIA), which confirmed the poor prognosis of patients with SLN metastases  $\geq 1$  mm in maximum diameter [27].

The Rotterdam criteria might be the most straightforward system, but it is also not without its challenges. For instance, it can be difficult to measure the diameter of curved lesions, and it is difficult to classify when 2 lesions are very close. Murali et al. performed a pivotal study that asked 7 expert pathologists to assess different SLN tumor burden criteria (Starz classification, micro-anatomic location and maximum diameter) in 44 positive SLNs and demonstrated that the highest inter-observer concurrence was for the maximum diameter [28].

Subsequently, adjuvant systemic therapy trials, such as the EORTC 18071 (high dose ipilimumab), EORTC 1325/Keynote 054 (pembrolizumab) and COMBI-AD (dabrafenib and trametinib) studies have used this 1 mm threshold as an inclusion criterion for AJCC stage IIIA patients (7th edition) [29–31]. Furthermore, Madu et al. demonstrated recently, that the inclusion of the 1 mm threshold in the new, 8th edition of the AJCC staging system would still help to improve survival discrimination within stage IIIA disease [32]. This is nowadays still of importance for both ongoing adjuvant therapy trials and to counsel and determine treatment for patients outside of clinical trials now that certain adjuvant therapies have been approved. Moreover, the EORTC 1208 (Minitub) study (NCT NCT01942603) is examining the biology of patients with tiny SLN metastases, with accrual expected to be completed in 2020.

## Conclusions

SLN tumor burden allows for stratification of positive SLN patients into cases with lower and higher risk disease. This is of importance to select patients for adjuvant systemic therapy or for entry into clinical trials. Until another biomarker can replace it, the maximum diameter of the largest lesion in the SLN, according to the Rotterdam/EORTC criteria, is the best and most reproducible way to assess SLN tumor burden.

## Should a completion lymph node dissection be done following a positive melanoma sentinel node biopsy?

Douglas Reintgen, MD

Extracted from the presentation of Merrick Ross, MD

Most patients diagnosed with Stage I and II melanoma are undergoing SLNB as their nodal staging procedure. Those with a positive SLN have several therapeutic options. Since 80–85% of patients with Stage III micrometastatic melanoma have nodal disease confined to the SLN, most positive SLN patients who are treated with a CLND would be exposed to the complications of a CLND when they cannot possibly benefit from the additional surgery. By intervening early with a CLND for microscopic nodal metastases, one prevents the development of clinical (palpable) disease in the basin and allows for better regional disease control. But only patients with non-SLN disease, which is estimated to occur in 10–20% of positive SLN cases, derive a benefit from CLND. Furthermore, patients with additional non-SLN disease beyond the any SLN metastasis have been shown to have worse survival, and therefore knowing the status of non-SLN disease potentially provided prognostic information. Two trials have addressed this issue, MSLT-2 and DeCOG-SLT [3, 4]. Both of these trials randomized patients with a positive SLN to either a CLND versus ultrasound surveillance and a TLND only if a nodal recurrence occurred. Both of the trials showed no significant survival benefit of performing a CLND versus nodal observation for a positive SLN. In addition, it is clear that controlled for the number of nodes involved, patients with disease beyond the SLN in the nodal basin do worse than patients with SLN only disease [33], but surgeons have to perform a CLND to obtain this information. Some institutions support a stratified approach to the question of when to offer a CLND after a positive SLNB. Most of these studies have suggested characteristics of the SLN, such as SLN disease volume, extranodal disease, and number of positive SLNs may guide the need for a CLND. With the approval of immune checkpoint inhibitors and targeted therapy for the adjuvant treatment of melanoma with lymph node metastases, CLND is not the last therapy option that can be offered to patients with a positive SLN. With early initiation of effective systemic adjuvant therapies, whether or not a CLND is performed after a positive SLN becomes less important. Systemic adjuvant therapy for melanoma with nodal metastases will be discussed in the following section by Drs. Lin Wang and Kevin Kim.

## Systemic adjuvant therapy for melanoma with regional nodal metastasis

Lin Wang, MD, PhD, Kevin B. Kim, MD

## Introduction

The incidence of melanoma continues to rise in the United States, with an estimated 100,350 new cases in 2020 [34]. Despite the increase in melanoma incidence, the estimated total number of deaths from melanoma has, fortunately, decreased for the past several years [13], likely due to a combination of early melanoma detection and successful development of novel systemic therapies for advanced melanoma. If diagnosed in the early stages, complete surgical excision with negative margins remains the best curative treatment for melanoma. If a patient is diagnosed with distant metastatic disease, systemic therapy including immunotherapy and/or targeted therapy is considered the mainstay of treatment. For patients with stage III melanoma with regional nodal metastasis, especially those with stage IIIB, IIIC or IIID (according to 8th edition AJCC), the risk of melanoma recurrence is relatively high even after complete surgical excision of the primary melanoma and regional nodal disease. Without effective adjuvant systemic therapies, 5-year recurrence-free survival (RFS) rates are approximately 38%, 30% and 18% in stage IIIB, IIIC and IIID melanoma, respectively [35]. For these patients, a combination of surgical excision and systemic therapy will be necessary to improve clinical outcomes.

High-dose interferon (IFN)- $\alpha$  was the first drug which was approved by the Food and Drug Administration (FDA) in the United States for use in an adjuvant setting, followed by pegylated IFN- $\alpha$ . With the development of checkpoint inhibitors, anti-cytotoxic T-lymphocyte associated protein (CTLA)-4, and anti-programmed cell death (PD)-1 antibodies, RFS and overall survival (OS) of patients with stage III melanoma have improved, and these novel

immunotherapies have also obtained FDA approval as adjuvant therapies. In addition, advanced understanding of genetic aberrations in melanoma has led to development of specific small-molecule inhibitors for mutated *BRAF* proteins, and a combination of BRAF and MEK inhibitors have shown clinical benefit in patients with a high recurrence risk, leading to FDA approval of targeted therapy in the adjuvant setting.

In this section, we will describe the rationales and results of pivotal adjuvant clinical trials that have changed the standard of care for melanoma patients with regional nodal metastasis. Table 1 lists FDA-approved systemic adjuvant therapies for node-positive melanoma. Table 2 summarizes the key data of individual clinical trials included in this review.

## Interferon- $\alpha$

IFN- $\alpha$  is a cytokine which has multiple important functions in the human body, including induction of immune modulation, stimulation of host rejection of tumor cells, and inhibition of angiogenesis, as well as direct growth-inhibitory effects at high doses [36]. It is the first cytokine shown to have clinical benefits in melanoma in the adjuvant setting.

In a phase III randomized trial (ECOG 1684), patients with primary melanoma thicker than 4 mm or regional lymph node involvement (stage III) were randomized to either 1 year of high-dose IFN- $\alpha$  treatment (20 million units [MU]/m<sup>2</sup> daily given intravenously for 5 days per week for 4 weeks, followed by 10 MU/m<sup>2</sup> 3 days per week by subcutaneous injection for 48 weeks) or close observation. In the original report, which had a median follow up duration of 6.9 years, there was a significant improvement

**Table 1** FDA-approved systemic adjuvant therapy regimens for nodal positive melanoma

Drug name	Dosing regimen	Indications
High-dose IFN- $\alpha$ -2b	20 MU/m <sup>2</sup> IV 5 days a week for 4 weeks, then 10 MU/m <sup>2</sup> SQ 3 times a week for 48 weeks	Lymph node metastasis (stage III) and/or > 4 mm Breslow thickness
Pegylated-IFN- $\alpha$ -2b	6 mcg/kg/week SQ for 8 doses, followed by 3 mcg/kg/week SQ for up to 5 years	Lymph node metastasis (stage III)
Ipilimumab	10 mg/kg IV every 3 weeks for 4 doses, followed by 10 mg/kg IV every 12 weeks for up to 3 years (alternatively, 3 mg/kg IV every 3 weeks for 4 doses, followed by 3 mg/kg IV every 12 weeks for 4 doses)	Lymph node metastasis (stage III)
Nivolumab*	240 mg IV Q2 weeks or 480 mg IV Q4 weeks for 1 year	stage III or IV melanoma
Pembrolizumab*	200 mg IV Q3 weeks or 400 mg IV Q6 weeks for 1 year	Stage III melanoma
Dabrafenib + Trametinib*	Dabrafenib 150 mg PO BID + Trametinib 2 mg PO daily for 1 year	Lymph node metastasis; V600E/K <i>BRAF</i> mutation (stage III)

MU million units, IV intravenous, SQ subcutaneous, PO oral, BID twice daily

\*Included in the 2020 NCCN-guideline

**Table 2** Results of randomized phase III clinical trials in adjuvant setting for nodal-metastatic melanoma

Study	No. pts	Treatment	Primary endpoint	RFS	OS	Gr $\geq 3$ Adverse events
Eggermont, et al. [44–46], NCT00636168 (EORTC 18071)	951	Ipilimumab (Ipi) vs placebo	RFS	HR 0.76 (95% CI 0.64–0.89); $p < 0.001$ Medium: Ipi 26 mo.; Placebo 17 mo	HR 0.72 (95.1% CI 0.58–0.88); $p = 0.001$ OS rate at 5 yr.: Ipi 65.4%; Placebo 54.5%	Ipi 54%; Placebo 26%
Tarhini, et al. [48], NCT01274338 (E1609)	1670	High-dose Ipi vs Low-dose Ipi vs High-dose IFN- $\alpha$	OS; RFS	Low-dose Ipi vs High-dose IFN- $\alpha$ HR 0.85 (99.4% CI 0.66–1.09); $p = 0.065$ High-dose Ipi vs High-dose IFN- $\alpha$ HR 0.84 (99.4% CI 0.65–1.09); $p$ , NS	Low-dose Ipi vs High-dose IFN- $\alpha$ HR 0.78 (95.6% CI 0.61–0.99); $p = 0.044$ High-dose Ipi vs High-dose IFN- $\alpha$ HR 0.88 (95.6% CI 0.69–1.12); $p$ , NS	Low-dose Ipi 37%; High-dose Ipi 58%; High-dose IFN- $\alpha$ 79%
Eggermon, et al. [51, 52], EORTC 1325 (KEYNOTE-054)	1019	Pembrolizumab (Pembro) vs placebo	RFS	HR 0.57 (98.4% CI 0.43–0.74); $p < 0.001$ RFS rate at 12 mo.: Pembro 75%; Placebo 61%	n/a	Pembro 15%; Placebo 3%
Weber, et al. [53], NCT02388906 (CheckMate-238)	906	Nivolumab (Nivo) vs Ipilimumab	RFS	HR 0.65 (97.56% CI 0.51–0.83); $p < 0.001$ RFS rate at 12 mo.: Nivo 71%; Ipi 61%	n/a	Nivo 14%; Ipi 46%
Long, et al. [35, 56], NCT01682083 (COMBI-AD)	870	Dabrafenib + Trametinib (Dab + Tram) vs placebo	RFS; OS	HR 0.47 (95% CI 0.39–0.58), $p < 0.001$ RFS rate at 3 yr.: Dab + Tram 58%; Placebo 39%	HR 0.57 (95% CI 0.42–0.79), $p = 0.0006$ OS rate at 3 yr.: Dab + Tram 86%; Placebo 77%	Dab + Tram 36%; Placebo 10%
Maio, et al. [61], NCT01667419 (BRIM8)	498	Vemurafenib (Vem) vs placebo	DFS	HR 0.80 (95% CI 0.54–1.18); $p = 0.26$ Median (cohort 2): Vem 23 mo.; Placebo 15 mo	n/a	Vem 57%; Placebo 15%

No. Pts number of patients, OS overall Survival, RFS recurrence-free survival, DFS disease-free survival, mo months, yr years, HR hazard ratio, CI confidence interval, p p-value, n/a not available

in both median RFS and median OS [37]. The median RFS in the IFN- $\alpha$  group was 1.72 years while that of the observation group was 0.98 years ( $p = 0.0023$ ). In addition, the median OS in the IFN- $\alpha$  group was 3.82 years while that of observation group was 2.78 years ( $p = 0.0237$ ). Based on this positive finding, IFN- $\alpha$  was approved by the FDA in 1996 as an adjuvant treatment in patients with thick ( $> 4$  mm Breslow thickness) primary melanoma or regional lymph nodal involvement.

However, in a subsequent randomized study (ECOG 1690), which randomized patients into three arms, high-dose IFN- $\alpha$  (20 MU/m<sup>2</sup> daily IV 5 days/week for 4 weeks, followed by 10 MU/m<sup>2</sup> by subcutaneous injection 3 days/week for 48 weeks), low-dose IFN- $\alpha$  (3 MU/m<sup>2</sup> by subcutaneous injection 3 days/week for 2 years), and close observation, there was no OS benefit of high-dose IFN- $\alpha$ . The estimated 5-year OS rate was 52% for the high dose IFN- $\alpha$  group, 53% for the low-dose IFN- $\alpha$  group, and 55% for the observation

group [38]. In addition, an updated analysis of ECOG 1684 demonstrated a loss of OS benefit of high dose IFN- $\alpha$  treatment over observation at a median 12.6 years of follow up [39]. Furthermore, IFN- $\alpha$  was associated with significant toxicities, including flu-like symptoms, fatigue, neutropenia/leukopenia, fever/chills, myalgia, anorexia, nausea/vomiting, liver toxicity (especially increased transaminases), and neurologic/neuropsychiatric disturbances. Grade 3 or 4 adverse events were observed in 78% of patients who were treated with the high-dose IFN- $\alpha$ .

To optimize the clinical efficacy and safety profile of IFN- $\alpha$  therapy, pegylated (PEG) IFN- $\alpha$  was developed, which has a longer half-life and lower immunogenicity. It was subsequently investigated in an adjuvant clinical trial. In a large randomized phase III study (EORTC 18991), 1256 patients with stage III melanoma were randomized, after lymphadenectomy of regional nodal metastasis, to either PEG-IFN- $\alpha$  treatment (induction 6 mg/kg/week for 8 weeks and then maintenance 3 mg/kg/week, for an intended total duration of 5 years) or close observation [40]. In this study, PEG-IFN- $\alpha$  treatment was associated with a statistically significant, but clinically modest, improvement in RFS (HR 0.82, [95% CI 0.71–0.96];  $p=0.001$ ), and no OS benefit. Because the study met the primary endpoint of RFS, PEG-IFN- $\alpha$  was approved by the FDA; however, due to the significant adverse events (grade 3 or 4 adverse events occurred in 44% of patients) and only a modest RFS benefit, this treatment has not been widely used in clinical practice. As a result, neither high-dose IFN- $\alpha$  nor PEG-IFN- $\alpha$  is recommended as an adjuvant therapy by the NCCN practice guidelines any more, especially with the availability of more effective immunotherapy in melanoma [41].

## Anti CTLA-4 antibody

CTLA-4 is a co-inhibitory receptor molecule present on T lymphocytes that suppresses the function of T-cells when it binds to its ligands, B7-1 and B7-2 molecules on the antigen presenting cells [42]. Anti CTLA-4 antibody causes a dissociation of CTLA-4 molecule from its ligands, which in turn allows B7 molecules to bind co-stimulatory CD28 molecules present on T-lymphocytes, leading to the activation of T-cells and improve anti-tumor T-cell functions.

Ipilimumab, an anti CTLA-4 antibody, was approved by the FDA as a systemic therapy for unresectable metastatic melanoma in 2011, on the basis of superior OS (HR 0.68,  $p<0.001$ ) over the comparator treatment, glycoprotein gp100 vaccine [43]. To evaluate clinical benefit of ipilimumab in an adjuvant setting, a large phase III randomized control trial was conducted (EORTC 18071) [44]. In this study, 951 patients with stage III (AJCC version 7) cutaneous melanoma who had undergone CLND were randomized

to receive either ipilimumab (10 mg/kg IV every 3 weeks for four doses, followed by every 3 months for up to 3 years) or placebo. Patients with stage IIIA melanoma with only 1 metastatic lymph node must have had nodal metastasis  $>1$  mm in size. In the ipilimumab treatment group, the RFS rate at 5 years was 40.8% compared to 30.3% in the placebo group (HR 0.76, [95% CI 0.64–0.89];  $p<0.001$ ). The OS rate at 5 years was 65.4% in the treatment group compared to 54.4% in the placebo group (HR 0.72, [95% CI 0.58–0.88];  $p=0.001$ ) [45]. These positive results led to FDA approval of high-dose ipilimumab as an adjuvant therapy in patients with stage III melanoma in 2015. In the most recent report of a long-term follow-up of the EORTC 18071 clinical trial, the RFS (HR 0.75, [95% CI 0.63–0.88];  $p<0.001$ ) and the OS (HR 0.73, [95% CI 0.60–0.89];  $p=0.002$ ) benefit in the ipilimumab group were durable at 7 years [46]. However, the high-dose ipilimumab treatment was associated with significant toxicity; Grade 3 or 4 adverse events were observed in 54% of patients, including 42% who experienced immune-related grade 3 or 4 adverse events [45]. The most common severe immune-related adverse events were diarrhea/colitis, hepatitis, dermatitis, hypophysitis, and lipase increase. Five patients (1.1%) died due to treatment-related toxicity: 3 died of colitis, 1 died of myocarditis, and 1 died of Guillain-Barre' syndrome.

Due to the risk of significant toxicity associated with the treatment, high-dose ipilimumab has to be used with great caution. Considering that the standard dose of ipilimumab for the treatment of unresectable metastatic melanoma is 3 mg/kg, a phase III study (ECOG 1609) was designed to compare the standard dose (3 mg/kg) of ipilimumab with the standard high-dose IFN- $\alpha$ , and, separately, the high-dose (10 mg/kg) ipilimumab therapy with the high-dose IFN- $\alpha$  treatment [48]. This study was a large open-label randomized phase III study in patients with resected cutaneous melanoma (stage IIIB, IIIC, M1a, or M1b per AJCC version 7) with the co-primary end points of RFS and OS. A total of 1,670 patients were randomized at a 1:1:1 ratio to ipilimumab 3 mg/kg (every 3 weeks for 4 doses, followed by 4 additional maintenance doses every 12 weeks), ipilimumab 10 mg/kg (every 3 weeks for 4 doses, followed by 4 additional maintenance doses every 12 weeks), or high-dose IFN- $\alpha$  treatment, and treated for 1 year. Ipilimumab 3 mg/kg was shown to be superior to high-dose IFN- $\alpha$  based on the OS (HR 0.78, [95% CI 0.61–0.99];  $p=0.044$ ) and RFS rates (HR 0.85, [95% CI 0.66–1.09];  $p=0.065$ ). However, Ipilimumab 10 mg/kg when compared with high-dose IFN- $\alpha$ , did not achieve statistical significance in terms of the primary endpoints. Moreover, the incidence of grade  $\geq 3$  adverse events was the lowest in the group of patients receiving ipilimumab 3 mg/kg: 37% in the lower dose ipilimumab group compared to 79% in the high-dose IFN- $\alpha$  group and 58% in the high-dose ipilimumab group. On the basis of

the efficacy and safety data of these two large randomized studies, ipilimumab at 3 mg/kg is preferred over 10 mg/kg in clinical practice, despite the fact that the FDA-approved dosing schedule of adjuvant ipilimumab is 10 mg/kg every 3 weeks for 4 doses, followed by 10 mg/kg maintenance every 12 weeks up to 3 years.

## Anti PD-1 antibody

PD-1 is another co-inhibitory immune-checkpoint molecule in cytotoxic T-cells. Upon binding to its ligands, PD-L1 (presented on tumor cells and other immune cells) and PD-L2, PD-1 causes suppression of T-cell functions [48, 49]. Anti PD-1 antibodies, such as nivolumab and pembrolizumab, block the binding of PD-1 to its ligands, and restores the lytic activity of cytotoxic T-cells against tumor cells.

In the metastatic setting, anti PD-1 antibody therapies have been shown to improve both progression-free survival (PFS) and OS when compared to either chemotherapy or ipilimumab [50, 51]. There is an approximately 46–47% decrease in the risk of disease progression (HR of 0.53–0.54 for PFS) and approximately 27–37% reduction in the risk of death (HR of 0.63–0.73 for OS) with either nivolumab or pembrolizumab compared to ipilimumab treatment. As a result, anti PD-1 antibody therapies have replaced ipilimumab as the preferred immunotherapy in patients with advanced melanoma. It is not surprising that these anti PD-1 antibody therapies have been promptly investigated in the adjuvant setting in melanoma. The EORTC 1325/Keynote 054 study was a phase III randomized, double-blind trial that evaluated the clinical benefit of pembrolizumab (200 mg IV every 3 weeks for 1 year) versus placebo in patients (total  $n=1019$ ) with stage III melanoma after complete regional lymphadenectomy [50]. Patients with stage IIIA (AJCC version 7) nodal micrometastasis measuring  $<1$  mm or in-transit metastases were excluded from the study. In the pembrolizumab treatment group, the one-year RFS rate was 75.4% compared to 61.0% in the placebo group (HR 0.57 [95% CI 0.43–0.74];  $p<0.001$ ). In a subgroup analysis, the benefit of pembrolizumab was observed regardless of PD-L1 status. In this study, treatment-related grade  $\geq 3$  adverse events occurred in 14.7% of patients who were treated with pembrolizumab, and there were no treatment-related deaths. Overall, 13.8% of patients discontinued pembrolizumab treatment due to adverse events. These findings led to the approval of pembrolizumab by the FDA for resected high-risk stage III melanoma in 2019.

Recent follow-up data for the EORTC 1325/Keynote 054 study demonstrated a continuing RFS advantage for pembrolizumab [51]. The 3-year RFS rates were 63.7% and 44.1% in the pembrolizumab and the placebo group, respectively (HR 0.56, [95% CI 0.47–0.68]). The 3-year

cumulative incidence of distant metastasis as the first recurrence was 22.3% in the pembrolizumab group vs 37.3% in the placebo group (HR 0.55 [95% CI 0.44–0.69]).

Nivolumab was also evaluated in the adjuvant setting in patients who were at a high-risk for melanoma recurrence or metastasis. Checkmate-238 was a phase III randomized double-blind study of nivolumab (3 mg/kg IV every 2 weeks up to 1 year) versus high-dose ipilimumab (10 mg/kg IV every 3 weeks for four doses followed by every 12 weeks), the standard at the time of the study design [52]. In this study, both stage III (except IIIA) and IV (AJCC version 7) patients were enrolled, and a total of 906 patients were randomized based on disease stages (stage IIIB, IIIC, IV M1a or M1b, IV M1c) and PD-L1 status, with the primary end point of RFS. Checkmate-238 was different from EORTC 1325/Keynote 054 or EORTC 18071 in that patients with resected stage IV disease were included. Similar to the EORTC 18071 and EORTC 1325/Keynote 054 studies, complete regional lymphadenectomy was required for stage III patients. Patients in the nivolumab arm had significantly longer RFS over those in the ipilimumab arm (HR 0.65, 95% CI 0.51–0.83);  $p<0.001$ ). The 1-year rate of RFS was 70.5% in the nivolumab arm and 60.8% in the ipilimumab arm. Immune-related adverse events were significantly less frequent and less severe with nivolumab treatment; Grade 3 or 4 toxicity occurred in 14.4% and 45.9% of patients who were treated nivolumab and ipilimumab, respectively. Accordingly, a lower percentage of patients in the nivolumab arm required treatment discontinuation due to the toxicity (9.7% versus 42.6%). These findings led to approval of nivolumab by the FDA for the treatment of resected stage III or IV melanoma with high risk for recurrence in December of 2017.

The benefit of nivolumab was sustained at a median follow up of 4 years. A recent update of Checkmate-238 reported superior 4-year RFS (51.7% [95% CI 46.8–56.3]) in the nivolumab group over ipilimumab (41.2% [95% CI 36.4–45.9]) with HR of 0.71 (95% CI 0.60–0.86,  $p=0.0003$ ) [53]. Based on these data, nivolumab is generally preferred over ipilimumab in an adjuvant setting.

It appears that the clinical benefit of nivolumab is similar to that of pembrolizumab, but there has not been a head-to-head comparison of these two anti-PD-1 antibodies. Since these adjuvant therapy trials were designed, additional dosing schedules of these 2 drugs have been approved by the FDA on the basis of the pharmacokinetic and safety profile data. At this time, the most commonly adapted dosing schedules include: nivolumab 240 mg IV every 2 weeks or 480 mg IV every 4 weeks for 1 year, and pembrolizumab 200 mg IV every 3 weeks or 400 mg IV every 6 weeks for 1 year.

## BRAF/MEK inhibitor

Approximately 50% of melanomas harbor an oncologic mutations in BRAF kinase, of which V600E and V600K accounts for nearly 95% [54]. These V600 BRAF mutation constitutively activates MEK and ERK proteins within the MAPK signal transduction pathway, leading to tumor cell proliferation, invasion and survival [54].

In patients with metastatic, unresectable, V600 BRAF-mutant melanoma, combinations of BRAF inhibitors and MEK inhibitors have shown significant OS improvement. In terms of V600 BRAF-targeting therapy, three regimens have been approved by the FDA: dabrafenib/trametinib, vemurafenib/cobimetinib and encorafenib/binimetinib. These regimens have been shown to reduce the risk of death by approximately 30–40% (HR of 0.61–0.70) when compared to BRAF inhibitor treatment alone [55–58].

The COMBI-AD trial was designed to address the hypothesis that a combination of BRAF and MEK inhibitors meaningfully prolongs RFS in patients with resected melanoma at high-risk for recurrence. This trial was a double-blind, phase III study that randomized 870 patients with stage IIIA (> 1 mm lymph node metastasis), IIIB or IIIC (AJCC v7) BRAF-V600E or V600K mutant melanoma to receive either a combination of dabrafenib (150 mg PO twice daily) and trametinib (2 mg PO daily) or placebo for 1 year [31]. The primary end point was RFS and OS, and distant-metastasis-free survival and safety were the secondary endpoints. At a median follow-up time of 2.8 years, patients in the treatment arm had significantly longer RFS compared with those with the placebo arm (HR 0.47, [95% CI 0.39–0.58];  $p < 0.001$ ). The 3-year RFS rate was 58% and 39% in the treatment arm and the placebo arms, respectively. Likewise, the 3-year OS rate was superior in the treatment group (86% versus 77%, HR 0.57, [95% CI 0.42–0.79];  $p = 0.0006$ ). In April of 2018, the FDA approved the combination of dabrafenib and trametinib for the treatment of resected stage III V600 BRAF-mutant melanoma. A recent long-term update of the COMBI-AD trial data confirmed the long-term benefit of adjuvant dabrafenib and trametinib at the 5-year analysis. This updated report showed that whereas the median RFS duration was 16.6 months in the placebo group, a median RFS had not been reached in the dabrafenib and trametinib treatment arm (HR 0.51, [95% CI 0.42–0.61]). The 5-year RFS rate was 52% ([95% CI 48–58%]) in the dabrafenib and trametinib group versus 36% ([95% CI 32–41%]) in the placebo group [59].

Grade 3 or 4 adverse events occurred in 41% of patients who were treated with the combination of dabrafenib and trametinib, and 26% of patients discontinued the treatment due to intolerable side effects. The most common adverse

events of the combination regimen were pyrexia, chills, fatigue, nausea, headaches and diarrhea, arthralgia and rash [31, 59].

BRIM8 was a phase III double-blind, randomized study, aiming to assess the clinical benefit of a single-agent BRAF inhibitor, vemurafenib, in the adjuvant setting [60]. In this clinical trial, 498 patients with stage IIC/IIIA/IIIB (cohort 1) or stage IIIC (cohort 2) melanoma were randomized to receive either vemurafenib (960 mg PO twice a day) or placebo for 1 year after complete melanoma resection, and for those with stage III disease, lymph node dissection. This study utilized a hierarchical analysis of cohort 2 before analysis of cohort 1. In cohort 2, patients with stage IIIC disease, the median disease-free survival (DFS) was 23.1 months in the vemurafenib group compared to 15.4 months in the placebo group (HR 0.80, [95% CI 0.54–1.18,  $p = 0.26$ ]). In cohort 1, for patients with stage IIC/IIIA/IIIB disease, median DFS was not reached in the treatment group, whereas median DFS was 36.9 months in the placebo group (HR 0.54, [95% CI 0.37–0.78]; log-rank  $p = 0.0010$ ). However, because of the study's original statistical design of pre-specified hierarchical analysis of cohort 2 data prior to cohort 1, the study was determined not to have met its primary endpoints. With the approval of the combination of dabrafenib and trametinib, vemurafenib treatment alone is not recommended in the adjuvant setting.

## Regimens under investigation for adjuvant therapy

In addition to the success and approval of multiple regimens for adjuvant systemic therapy in resected melanoma in recent years, the field of melanoma therapy continues to evolve with many promising options under investigation. One of them is the combination of anti PD-1 and anti CTLA-4 inhibitors. Nivolumab in combination with ipilimumab was previously demonstrated to be superior to ipilimumab alone in prolonging PFS and improving OS in patients with treatment-naïve metastatic melanoma [61, 62]. A clinical trial, CheckMate 915 (NCT03068455), was designed to explore clinical benefit of the combination of nivolumab and ipilimumab in the adjuvant setting. It was a phase III, randomized, placebo-controlled, double-blind trial of adjuvant immunotherapy with nivolumab (240 mg IV every 2 weeks) combined with Ipilimumab (1 mg/kg IV every 6 weeks) for 1 year versus nivolumab monotherapy in patients with completely resected melanoma (stage IIIB-IV). The primary end point was RFS, and accrual was completed in 2019. Unfortunately, the sponsor of the study announced in a press release that the study did not meet the primary endpoint of significant RFS benefit of the combination in PD-L1 negative melanoma or in all intent-to-treat patients



regardless of PD-L1 status. A detailed report of the study results is pending this time.

Interleukin-2 (IL-2) stimulates T-cell activation and proliferation, and is approved for the treatment of metastatic melanoma. However, IL-2 treatment is associated with significant toxicity and leads to meaningful clinic benefit in only a small percentage of patients. A recently developed IL-2 cytokine prodrug, bempedaldesleukin (NKTR-214), has been shown to have anti-tumor immunity in various tumor models including melanoma. In vivo, bempedaldesleukin increased survival of effector T-cells and depleted regulatory T-cells, and a combination of anti PD-1 inhibitor with bempedaldesleukin had synergistic anti-tumor activity in various tumor types [63]. Currently, a phase III randomized study is underway comparing the efficacy of bempedaldesleukin in combination of nivolumab with that of nivolumab alone in the adjuvant setting in patient with stage IIIB/C/D (AJCC version 8) or stage IV melanoma (NCT04410445). RFS will be the primary endpoint, and OS will be one of the secondary outcome measures.

Another strategy for systemic adjuvant therapy is a vaccine approach. It is not new to utilize vaccine-induced anti-tumor immune response as a therapeutic tool for cancer therapy. In melanoma, there are various types of melanoma vaccines under exploration for adjuvant therapies, owing to the progress in our knowledge of tumor immunology. Two such studies in vaccine development employed the strategy of using predicted immunopeptides, based on known melanoma genome mutations, to design personalized vaccines [64, 65]. Both studies showed that neoepitopes elicit T cell anti-tumor activity in vivo. Combining the neoepitope vaccine with an anti PD-1 antibody further boosted the anti-tumor immune response. This led to the KEYNOTE-942 clinical trial, a randomized, open-label, phase II study to assess the efficacy of adjuvant mRNA-4157 in combination with pembrolizumab versus pembrolizumab alone in patients with stage IIIB/C/D and stage IV melanoma after

resection of melanoma with or without lymph node dissection (NCT03897881). RFS is the primary endpoint, and this study is currently underway. Another ongoing study is a phase III, double-blind, placebo-controlled clinical trial of seviprotimut-L (NCT01546571), which is a partially purified shed melanoma antigens vaccine derived from three human melanoma cell lines. Although RFS was significantly prolonged with the intradermal seviprotimut-L vaccine treatment compared to placebo in patients over age of 60 or those with stage IIB/IIC melanoma, unfortunately, RFS was not significantly improved in the vaccine treatment arm by intent-to-treat analysis for the full study population (AJCC v7 stage IIB/IIC/III melanoma) [66, 67]. Table 3 lists information about ongoing investigational clinical trials.

## Conclusion

Over the past several years, with the development of more effective adjuvant therapies, both RFS and OS have improved in melanoma patients with node-positive metastasis. In addition to superior clinical efficacy, these newer therapies are better tolerated with lower incidence of severe adverse events. Despite these advances in adjuvant therapy, there are still a number of ongoing questions. A substantial portion of patients with stage IIIB-IIID disease still develop melanoma recurrence within 5 years. We will need to continue to develop more effective novel therapies with strong scientific bases. We can investigate whether the combination of a BRAF-targeting drug and a checkpoint inhibitor is superior to either therapeutic modality alone in patients with *V600 BRAF*-mutant melanoma, as it has shown to be in patients with unresectable advanced melanoma [68]. It would also be of clinical significance to identify more robust biomarkers to better predict the long-term clinical benefits of checkpoint inhibitors and/or targeted therapies, which will allow us to tailor regimens to different groups of patients

**Table 3** Currently enrolling or recently completed randomized adjuvant therapy studies

Clinical trial	Treatment	Patient population*	Primary endpoint	No. of patients	Status
NCT03068455 (CheckMate-915)	Nivolumab + Ipilimumab vs Nivolumab + Placebo	Stage IIIB/C/D-IV**	RFS	1943	Completed accrual
NCT03897881 (KEYNOTE-942)	mRNA-4157 + Pembrolizumab vs Pembrolizumab alone	Stage IIIB/C/D-IV**	RFS	150	Phase 2, recruiting
NCT04410445	Bempedaldesleukin + Nivolumab vs Nivolumab alone	Stage IIIA(LN metastasis > 1 mm), IIIB/C/D-IV**	RFS	950	Recruiting
NCT01546571	Seviprotimut-L vs placebo	Stage IIB/C-III*	RFS	1224	Completed accrual

OS overall survival, RFS recurrence-free survival, PFS progression-free survival, LN lymph node

\*According to the 7th edition of AJCC staging; \*\*according to the 8th edition of AJCC staging

to achieve better outcome and lower incidence of adverse events.

## Neoadjuvant systemic therapy for clinical stage III melanoma

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Clinically detectable stage III melanoma is a challenging and heterogeneous disease. Standard management is wide local excision of the primary lesion (when present) with TLND, followed by adjuvant systemic therapy with or without radiation to the involved nodal basin [41]. Initially indicated for patients with advanced, unresectable disease, immune mediated (anti-cytotoxic T lymphocyte-4 [anti-CTLA-4] and anti-programmed death-1 [anti-PD-1]) and BRAF/MEK-pathway targeted molecular therapies have revolutionized the modern landscape of melanoma treatment, and since 2015 have been approved for use in the adjuvant setting for stage III melanoma [2–11, 32]. The role of systemic adjuvant therapy for melanoma with regional nodal metastases was reviewed and discussed in the prior section by Lin Wang and Kevin Kim. Neoadjuvant therapy can offer several advantages over adjuvant regimen approaches and is routinely used for several solid malignancies, including breast, esophageal, anal, rectal and bladder cancers [12, 69–71]. Multiple phase I and II clinical trials evaluating neoadjuvant immune checkpoint blockade and targeted molecular therapy for clinical stage III melanoma have yielded promising results, and several more are ongoing [72–79]. Many of these studies vary in their study design and measured endpoints; the International Neoadjuvant Melanoma Consortium (INMC) was developed in an effort to standardize optimal trial designs and outcomes for future trials [80].

### The utility of neoadjuvant therapy

Early evidence for the benefit of neoadjuvant therapy was based on animal models of metastatic breast and lung cancers which demonstrated an increase in circulating growth factors and accelerated enlargement of distant tumor foci following removal of the primary tumor [81]. Administration of chemotherapy or radiation therapy prior to tumor resection reduced the concentration of these circulating growth factors and markedly slowed the growth of these residual tumor deposits after excision of the primary tumor [82]. Additional studies, investigating the mechanisms and effects of immune mediated therapy, have demonstrated that after neoadjuvant administration of these agent, there is a measurable increase in circulating tumor specific antigens released from the primary malignancy. These released antigens then prime the

host's immune system, inciting a more robust immune-mediated response to residual disease [83].

Theoretical advantages of a neoadjuvant approach include tumor downsizing (allowing for less extensive surgery), assessment of tumor biology and better patient selection, and evaluation of in-vivo sensitivity to therapy. Much insight can be gained by evaluating the response of the primary tumor to neoadjuvant treatment. The degree of tumor response can also be quantified on pathologic review, and additional treatment tailored based on the extent of this pathologic response. Ample data exists in the breast cancer literature demonstrating improved long-term outcomes for patients with a complete pathologic response (pCR) after treatment, defined as a complete absence of viable tumor cells within the reviewed specimen [84, 85]. With neoadjuvant immunotherapy, response rates of 9–73%, with rates varying widely depending on the regimen given, have been reported while response rates of approximately 85% are seen with targeted therapy (Table 4).

Collection of peripheral blood and tissue samples at the time of diagnosis can provide invaluable biospecimens for translational research. Studying the effects of treatment on these specimens can lead to discovery of predictive and prognostic biomarkers, improved understanding of drug mechanisms of action, and identification of patterns of tumoral resistance to therapy, all of which can help guide adjuvant treatment regimens and provide novel therapeutic targets [86–88]. These prospective benefits must be balanced with the potential risks of neoadjuvant therapy, including the development of significant drug-induced toxicities and/or disease progression which could result in the inability to perform definitive surgical intervention.

### Evidence for neoadjuvant therapy in stage III melanoma

Several neoadjuvant agents have been studied for the treatment of clinical stage III melanoma, including high dose interferon, chemotherapy, biochemotherapy, and even oncolytic viral therapy. Over the last decade, investigations have largely focused on the novel classes of immune checkpoint inhibitors and targeted molecular therapies.

### Immune mediated and targeted molecular therapy

Melanoma is an immunogenic tumor, making immune mediated therapy an intuitive option for all stages of disease [47]. T-cell mediated responses to melanoma induce a shift in helper T-cells from a Th2 to Th1 population, promoting cytotoxic T-lymphocyte activity against the primary lesion and reducing disease progression [80–90]. These molecular reactions are characterized in part by the presence of tumor infiltrating lymphocytes (TILs), which when present

**Table 4** Completed trials evaluating neoadjuvant immune checkpoint and targeted molecular therapy for treatment of clinically-evident, resectable stage III melanoma

Investigator; year	Trial design	Patient #; stage disease	Treatment regimen	Findings
<i>Immunotherapy trials</i>				
Tarhini et al. (2014)	Phase I; single arm	33; stage IIIB/C	A. Ipilimumab 10 mg/kg every 3 weeks for 2 cycles→surgery at week 6–8→ipilimumab 10 mg/kg every 3 weeks for 2 cycles starting 2–4 weeks after surgery	<ul style="list-style-type: none"> <li>• 9% RR</li> <li>• 0% pCR</li> <li>• 10.8 month PFS</li> <li>• 42% grade 3 toxicities; no grade 4/5 toxicities</li> <li>• ↑ Tregs and ↓ MDSCs in periphery correlated w/ improved PFS</li> <li>• ≥ threefold ↑ CD3<sup>+</sup>/CD4<sup>+</sup>/IFN<math>\gamma</math><sup>+</sup> T-cells seen only in those with PFS at 6 months</li> <li>• 29% RR with 3 mg/kg vs. 43% RR w/ 10 mg/kg</li> <li>• 36% pCR with 3 mg/kg vs. 29% pCR w/ 10 mg/kg</li> <li>• 10/11 patients with pCR or MRD remained disease free at 32 months</li> <li>• More grade 3/4 toxicities with higher ipilimumab dosing; no grade 5 toxicities</li> <li>• ↑ TILs in TME seen with pCR, regardless of ipilimumab dosing</li> <li>• ↑ baseline and post-neoadjuvant TIL clonality correlated w/ improved RFS</li> </ul>
Tarhini et al. (2018)	Phase I; double arm	30; stage IIIB/C	A. Ipilimumab 3 mg/kg every 3 weeks for 2 cycles→ surgery at week 6–8→ Ipilimumab 3 mg/kg every 3 weeks for 2 cycles B. Ipilimumab 10 mg/kg every 3 weeks for 2 cycles→ surgery at week 6–8→ Ipilimumab 10 mg/kg every 3 weeks for 2 cycles * Standard HDI given to both groups neoadjuvantly and adjuvantly	<ul style="list-style-type: none"> <li>• 25% RR in mono group vs. 73% RR in combo group</li> <li>• 25% pCR in mono group vs. 45% pCR in combo group</li> <li>• PFS, RFS, DMFS, and OS favored combo group</li> <li>• Disease progression prevented surgery in 2 patients in mono group, resulting in early cessation of trial</li> <li>• 73% toxicities and 64% dose delays in combo group</li> <li>• ↑ CD8<sup>+</sup> TILs, PD-L1 expression, and TCR clonality in treatment responders</li> <li>• 29% pCR or near major pathologic response</li> <li>• 63% DFS and 93% OS rate at 2 years; all patients with pathologic response were disease free</li> <li>• No grade 3 or higher toxicities; no surgical delays</li> <li>• ↑ Tex cells in periphery and in tumor at resection associated with improved outcomes</li> <li>• Potential neoadjuvant response signal identified</li> </ul>
Amaria et al. (2018)	Phase II; double arm	23; stage IIIB/C, and oligometastatic stage IV	A. Nivolumab 3 mg/kg every 2 weeks for 4 cycles→ surgery→ nivolumab 3 mg/kg every 2 weeks for 13 cycles B. Ipilimumab 3 mg/kg + nivolumab 1 mg/kg every 3 weeks for 3→ surgery→ nivolumab 3 mg/kg every 2 weeks for 13 cycles	<ul style="list-style-type: none"> <li>• 25% RR in mono group vs. 73% RR in combo group</li> <li>• 25% pCR in mono group vs. 45% pCR in combo group</li> <li>• PFS, RFS, DMFS, and OS favored combo group</li> <li>• Disease progression prevented surgery in 2 patients in mono group, resulting in early cessation of trial</li> <li>• 73% toxicities and 64% dose delays in combo group</li> <li>• ↑ CD8<sup>+</sup> TILs, PD-L1 expression, and TCR clonality in treatment responders</li> <li>• 29% pCR or near major pathologic response</li> <li>• 63% DFS and 93% OS rate at 2 years; all patients with pathologic response were disease free</li> <li>• No grade 3 or higher toxicities; no surgical delays</li> <li>• ↑ Tex cells in periphery and in tumor at resection associated with improved outcomes</li> <li>• Potential neoadjuvant response signal identified</li> </ul>
Huang et al. (2019)	Phase I; single arm	29; stage IIIB/C, and oligometastatic stage IV	A. Pembrolizumab 200 mg x 1 cycle→ surgery at week 3→ pembrolizumab 200 mg every 3 weeks for 1 year	<ul style="list-style-type: none"> <li>• 25% RR in mono group vs. 73% RR in combo group</li> <li>• 25% pCR in mono group vs. 45% pCR in combo group</li> <li>• PFS, RFS, DMFS, and OS favored combo group</li> <li>• Disease progression prevented surgery in 2 patients in mono group, resulting in early cessation of trial</li> <li>• 73% toxicities and 64% dose delays in combo group</li> <li>• ↑ CD8<sup>+</sup> TILs, PD-L1 expression, and TCR clonality in treatment responders</li> <li>• 29% pCR or near major pathologic response</li> <li>• 63% DFS and 93% OS rate at 2 years; all patients with pathologic response were disease free</li> <li>• No grade 3 or higher toxicities; no surgical delays</li> <li>• ↑ Tex cells in periphery and in tumor at resection associated with improved outcomes</li> <li>• Potential neoadjuvant response signal identified</li> </ul>

Table 4 (continued)

Investigator; year	Trial design	Patient #; stage disease	Treatment regimen	Findings
Blank et al. (2018)	Phase I; double arm	20; stage IIIB/C	<p>A. Ipilimumab 3 mg/kg + nivolumab 1 mg/kg every 3 weeks for 2 cyclesà surgery at week 6à ipilimumab 3 mg/kg + nivolumab 1 mg/kg every 3 weeks for 2 cycles</p> <p>B. Surgeryà ipilimumab 3 mg/kg + nivolumab 1 mg/kg every 3 weeks for 2 cycles</p>	<ul style="list-style-type: none"> <li>• 66% pCR or near pCR in neoadjuvant arm</li> <li>• 80% in neoadjuvant vs. 60% in adjuvant group were relapse free at 25.6 months</li> <li>• All patients w/ pathologic response relapse free at 25.6 months</li> <li>• 90% in each group stopped treatment early or had delays due to toxicities</li> <li>• ↓ TILs and ↓ T cell clonality associated w/ relapse</li> <li>• Low baseline IFN<math>\gamma</math> RNA expression in tumor associated w/ relapse</li> <li>• RR in 63% of arm A, 57% of arm B, and 35% of arm C</li> <li>• Pathologic response in 80% of arm A, 77% of B, and 65% of arm C</li> <li>• No relapse in patients w/ pathologic response</li> <li>• Grade 3/4 toxicities in 40%, 20% and 50% in arms A, B, and C, respectively</li> <li>• Surgery delayed for 1 patient in arm A and 2 in arm C</li> <li>• IFN<math>\gamma</math> RNA expression level did not correlate w/ outcome</li> </ul>
Rozeman et al. (2019)	Phase II; triple arm	86; stage IIIB/C	<p>A. Ipilimumab 3 mg/kg + nivolumab 1 mg/kg for 2 cyclesà surgery at 6 weeks</p> <p>B. Ipilimumab 1 mg/kg + nivolumab 3 mg/kg for 2 cyclesà surgery at 6 weeks</p> <p>C. Ipilimumab 3 mg/kg for 2 cyclesà nivolumab 3 mg/kg for 2 cyclesà surgery at 6 weeks</p>	
<i>Targeted therapy trials</i>				
Amaria et al. (2018)	Phase II; double arm	21; stage IIIB/C, and oligometastatic stage IV with BRAF mutation	<p>A. Dabrafenib 150 mg PO qd + trametinib 2 mg PO qd for 8 weeksà surgeryà Dabrafenib 150 mg PO qd + trametinib 2 mg PO qd for 44 weeks</p> <p>B. Surgeryà standard of care adjuvant therapy</p>	<ul style="list-style-type: none"> <li>• 71% of standard care arm developed negative survival events by 2 months, prompting early trial cessation</li> <li>• Median event free survival of 19.7 months vs. 2.9 months for neoadjuvant vs. standard of care arms</li> <li>• 85% RR and 75% pathologic response in neoadjuvant arm</li> <li>• Low pERK in baseline tumor specimens associated with pCR</li> <li>• Decreased pre-treatment T cell clonality associated with non-pCR</li> </ul>

**Table 4** (continued)

Investigator; year	Trial design	Patient #; stage disease	Treatment regimen	Findings
Long et al. (2019)	Phase II; single arm	35; stage IIIB/C with BRAF mutation	A. Dabrafenib 150 mg PO qd + trametinib 2 mg PO qd for 12 weeks à surgery à Dabrafenib 150 mg PO qd + trametinib 2 mg PO qd for 40 weeks	<ul style="list-style-type: none"> <li>• 86% RR</li> <li>• 100% pathologic response with 49% achieving pCR</li> <li>• 57% recurrence rate at 27 months</li> <li>• Median RFS of 30.6 months for pCR vs. 18 months for non-pCR</li> <li>• ↑ CD8<sup>+</sup> TILs and PD-L1 expression at baseline associated with pCR</li> <li>• Surgical resection easier in 46% of patients</li> <li>• 29% grade 3/4 toxicities</li> <li>• No correlation between ctDNA and RR or pathologic response</li> </ul>

correlate with improved DFS and OS [47, 89–91]. Functioning as monoclonal antibodies, immune checkpoint inhibitors block PD-1 (nivolumab and pembrolizumab) and CTLA-4 (ipilimumab) to downregulate host immune system inhibitory signals, thereby allowing for a more robust anti-tumor immunogenic response [92].

Up to 50–60% of patients with cutaneous melanoma harbor oncogenic point mutations in the *BRAF* gene [93, 94]. The product of *BRAF*, a serine/threonine kinase, leads to constitutive activation of the mitogen-activated protein kinase (MAPK) pathway, resulting in increased production of immunosuppressive factors by malignant melanoma cells, thus allowing the cancer to evade the host immune system and proliferate [95]. MAPK kinase (MEK) is an intermediary serine/threonine kinase, integral to the progression of the MAPK pathway [96]. Dabrafenib, a BRAF inhibitor, and trametinib, a MEK inhibitor, have both demonstrated improved survival outcomes when administered to patients with advanced stage BRAFV600 mutated melanoma, both as monotherapies or as combination therapy [97–99].

Results of the most recent major trials evaluating neoadjuvant immune checkpoint inhibitors and targeted molecular therapies for patients with clinical stage III melanoma are summarized in Table 4. Many of these trials involve combinations of therapies and multiple dosing schedules, allowing for identification of the optimal treatment regimen that will provide maximal therapeutic benefit while limiting toxic side effects. Although there were documented instances, it was exceedingly rare that patients in these trials developed disease progression on neoadjuvant treatment that delayed or inhibited surgical intervention. Very few patients suffered severe toxicities, and most toxicities were able to be managed effectively with routine care. In general, higher doses led to higher rates of toxicity, especially with ipilimumab.

Radiographic and pathologic responses were common endpoints in all conducted trials. Radiographic response tended to correlate modestly with pathologic response but was not consistently associated with improved outcomes. Many of the studies documented high rates of pathologic response following neoadjuvant treatment with both immune mediated and targeted molecular therapy. Unlike radiographic response, pathologic response had stronger associations with improved outcomes, with the greatest correlations being seen in patients who had a pCR.

Central to all trials was evaluation of the immunologic and biochemical effects induced by the treatment. Increases in TILs and T-cell receptor clonality were commonly associated with higher rates of pathologic response and improved prognosis, while decreases in regulatory T-cells correlated with worse outcomes. Among patients treated with targeted molecular therapies, lower levels of pERK at baseline and following treatment were associated with pCR. Other trials identified biomarkers indicative of

beneficial responses to neoadjuvant treatment, such as a signal composed of genes involved in T-cell activation, adaptive immune response, and T-cell migration that, when present, was associated with increased post-treatment TILs and RFS and demonstrated a potential screening marker to identify ideal treatment candidates. Conversely, lower levels of IFN $\gamma$  RNA expression in baseline tumor specimens were found to be potentially associated with poorer treatment response and higher rates of relapse.

### International neoadjuvant melanoma consortium guidelines

The currently published neoadjuvant therapy trials suffer from heterogeneous patient cohorts, inconsistent trial designs and endpoints, and small sample sizes, all of which reduce the ability to compare and generalize results. The INMC was founded to address these concerns, with the goal of establishing a standardized approach to the investigation of neoadjuvant systemic therapy for resectable, clinically stage III melanoma. In order to ensure that these benchmarks are met, the INMC has published recommendations focused around three core principles of trial design: patient selection criteria and treatment duration, trial endpoints, and biospecimen collection and translational research [81].

Patient enrollment should be restricted to those with clinically detectable, surgically resectable, stage III melanoma based on the AJCC 8th edition guidelines, with in-transit or resectable oligometastatic stage IV disease being evaluated as separate cohorts [100]. Neoadjuvant therapy should be limited to 6–8 weeks of treatment, allowing for the benefit of extended treatment pre-operatively but limiting the risk of disease progression to an unresectable stage. Trial endpoints should include safety, radiographic response, pathologic response, and survival outcomes. Computed tomography (CT) imaging with intravenous contrast should be obtained at baseline and after completion of neoadjuvant therapy, just prior to surgical resection. Following surgery, routine body and central nervous system surveillance imaging should be obtained every 3 months for the first 2 years, then every 6 months for up to 5 years, and yearly after that. Survival outcomes should include 1-year, 2-year, and 3-year assessments of RFS, event-free survival, distant metastasis-free survival, and OS and melanoma-specific survival. All trials should routinely collect biospecimens to facilitate translational research. Tumor sampling should be obtained at baseline, during the early neoadjuvant treatment phase, at the time of surgical resection, and at the time of relapse should it occur. Peripheral blood sampling should parallel tissue sampling.

### Ongoing and future trials

Several trials evaluating a multitude of neoadjuvant therapy regimens for resectable, clinical stage III melanoma are currently underway, and a few are briefly mentioned here [101]. Immunotherapy remains prevalent in a number of these studies but is frequently combined with additional modalities. In the PRADO extension of the OpACIN-neo trial, each patient's pathologic response to neoadjuvant therapy will be used to guide subsequent surgical and systemic treatment regimens [102]. Receptor tyrosine kinase inhibitors targeting vascular endothelial growth factor receptors (VEGFR) and histone deacetylase (HDAC) inhibitors can enhance the effects PD-1 inhibitors when paired together [103, 104]. Novel checkpoint inhibitors have shown promising pre-clinical results, and combinations of BRAF/MEK inhibitors with immunotherapy are being studied in patients with BRAF mutated melanoma [105, 106]. Finally, oncolytic viral therapies have proved efficacious in combination with checkpoint inhibitors in advanced melanoma, and may prove to be beneficial for patients with resectable, locally advanced disease [107, 108]. Results of these early trials are eagerly anticipated to determine whether these novel drugs and therapy combinations will provide robust responses and better outcomes.

### Conclusion

Use of neoadjuvant therapy for clinical stage III melanoma can potentially reduce the extent of surgical resection, improve long-term survival outcomes, and provide novel biomarkers that carry important prognostic implications. Moreover, complete response rates of 25–66% for immunotherapy and approximately 50% targeted therapy can be achieved (Table 4). Initiation of treatment with immunologic agents prior to surgical excision can prime the host immune system, potentially leading to a more robust response to adjuvant treatment. Combination therapy targeting multiple oncologic pathways may prove to be superior to monotherapy. pCR might ultimately prove to be a surrogate marker for treatment success, but its role as a prognostic marker needs to be further validated in large prospective randomized trials. Recent data suggests that while targeted therapy may induce higher rates of pCR, having a pCR following treatment with immunotherapy may be associated with more durable RFS [109]. Numerous clinical trials are ongoing, and all should strive to adhere to a consistent set of guidelines to ensure standardization in study design and outcome measures, and interpretability of results for the general melanoma population.

## Summary

Douglas Reintgen MD

Not all positive SLN metastases can be considered the same prognostically. The SLN is not a simple positive or negative result, and there is a gradient from very tiny to very large SLN tumor burden, with increasing risks. Dr. Alexander C.J. van Akkooi, MD, PhD from the Netherlands Cancer Institute emphasizes this fact and lists variables that have been used to predict prognosis based on SLN histopathology. These tumor burden factors include (a) a threshold of 2 and 3 mm micrometastases, (b) infiltration from the capsule, (c) surface area of node involved, (d) micrometastases location and (e) extracapsular extension. He concludes that the Rotterdam criteria, consisting of the largest diameter of the largest micrometastases is probably the most frequently used SLN tumor burden parameter. The Rotterdam criteria has demonstrated that increasing SLN tumor burden corresponds to worse survival and higher chance for non-SLN metastases in the CLND. Douglas Reintgen, MD discussed the role of CLND after a positive SLNB. He concluded that although 2 prospective randomized trials have shown no benefit for a CLND over ultrasound nodal surveillance, patients whose SLN has increased tumor volume, extracapsular disease, increased number of involved SLNs may warrant consideration for a CLND.

Drs. Wang and Kim discussed the development of more effective adjuvant therapies for clinical stage III melanoma, with both RFS and OS improving in patients with node-positive metastasis. In addition to superior clinical efficacy, these newer therapies are better tolerated and have a lower incidence of severe adverse events. In a discourse of the emerging use of neoadjuvant therapy for the treatment of stage III melanoma, Karakousis and colleagues state that immune mediated (anti-cytotoxic T lymphocyte-4 [anti-CTLA-4] and anti-programmed death-1 [anti-PD-1]) and BRAF/MEK-pathway targeted molecular therapies have revolutionized the modern landscape of melanoma treatment, and since 2015 have been approved for use in the adjuvant setting for stage III melanoma. Theoretical advantages of a neoadjuvant approach include tumor downsizing, allowing for less extensive surgery, assessment of tumor biology and better patient selection, and evaluation of in-vivo sensitivity to therapy. Like in breast cancer it is hoped that patients with complete pathologic response will have a better survival. The International Neoadjuvant Melanoma Consortium has recommended that (a) patient enrollment should be restricted to patients with resectable Stage III melanoma with other Stage III groups being studied separately, (b) limiting neoadjuvant therapy to 6–8 weeks, (c) having trial endpoints

to include safety, radiologic and pathologic response and survival data and (d) including serial tumor and peripheral blood sampling for a biorepository for future studies.

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## Declarations

**Conflict of interest** The authors have the following conflicts of interest to disclose. Kevin B. Kim has received honoraria from Genentech Inc., Novartis, Bristol-Myers Squibb, Regeneron and Sanofi for serving as a member of advisory board meeting and honoraria from Bristol-Myers Squibb, Novartis, Merck, Regeneron and Sanofi for serving as a member of speaker's bureau.

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